Physiol. Res. 52: 61-66, 2003

Serum Ghrelin Levels in Obese Patients: The Relationship to Serum Leptin Levels and Soluble Leptin Receptors Levels

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Received March 18, 2002
Accepted May 27, 2002

Summary

Ghrelin is a new endogenous ligand for the growth hormone secretagogue receptor. It activates the release of growth hormone from the pituitary and it also participates in the regulation of energy homeostasis. The aim of the study was to characterize changes in serum ghrelin levels in obese subjects and their relationship to the serum levels of leptin and soluble leptin receptor. Eight obese patients (6 women and 2 men) with body mass index (BMI) 40.3±13.4 $kg.m^{-2}$ and eight healthy controls (5 women and 3 men) with BMI 22.7±1.3 $kg.m^{-2}$ were examined. The ghrelin serum levels (165.0±58.1 vs. 343.37±81.96; $p<0.001$) and soluble leptin receptor serum levels (7.25±3.44 vs. 21.80±4.99; $p<0.0001$) were significantly lower in obese patients. The leptin serum levels (23.45±12.90 vs. 6.41±2.96; $p<0.005$) were significantly higher compared to the lean subject group. In both measured groups the levels of serum leptin significantly positively correlated with BMI. We proved a significantly lower serum ghrelin levels in the group of obese patients in comparison with the control group.

Key words

Ghrelin • Leptin • Soluble leptin receptor • Neuropeptide Y • Obesity

Introduction

Ghrelin is a new endogenous peptide, discovered in 1999 by Kojima and coworkers (Kojima *et al.* 1999, Hosoda *et al.* 2000; for review see Rosická *et al.* 2002). This peptide is composed of 28 amino acids with a unique octanoyl modification of hydroxy group on serine at the third position (Bednarek *et al.* 2000) that is essential for its function. Ghrelin is usually produced by so called Gr cells that are part of the endocrine system of the digestive tract (Date *et al.* 2000). It was first isolated from oxyntic mucosa of the stomach, which appears to produce ghrelin at much higher levels than other parts of the digestive tract, but it was also detected in the central nervous system, in kidneys, in the heart, in the parathyroid glands etc. (Papotti *et al.* 2000). Ghrelin is a strong stimulator of the growth hormone secretion in the somatotroph cells of hypophysis (Takaya *et al.* 2000, Peino *et al*. 2000). Studies in animal models indicate that this peptide hormone also plays an important role in signaling hypothalamic centers which regulate feeding and caloric state. The investigation carried out in the rodents demonstrated that the intracerebral and peripheral administration of ghrelin leads to an increase of food

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intake and a decrease of energy expenditure (Nakazoto *et al.* 2001). In addition, ghrelin is a potent stimulator of gastric motility and gastric acid secretion (Asakawa *et al.* 2001). Its long-term administration leads to a dose-dependent increase in body weight and ultimately to obesity (Wren *et al.* 2000). On the contrary, significantly lower plasma ghrelin concentration was observed in human obese subjects compared to lean subjects (Tschop *et al.* 2000).

Leptin is a protein hormone produced predominantly by the adipocytes. It is suggested that serum leptin concentrations represent an important peripheral signal that plays a role in the regulation of food intake and energy expenditure (Ahima *et al.* 1996). It causes the increased energy expenditure and the decreased food intake. Significant changes in serum leptin concentration were observed in the states accompanied by marked changes in body fat content. Serum leptin levels are therefore higher in obese patients (Maffei *et al*. 1995) and lower in malnutrition states and patient with anorexia nervosa (Haluzík *et al.* 1999a). Leptin effect is therefore antagonistic to the ghrelin effect. In ob/ob mice defective for leptin, which are monstrously fat, hyperfagic and infertile, a significantly higher ghrelin expression in stomach cells and higher plasma levels were observed in comparison with healthy mice. After leptin administration the ghrelin levels significantly decreased and simultaneously the reduction of food intake and energy expenditure was recorded. A significant decrease of ghrelin levels was also observed after the leptin administration to mice without the defective leptin gene (Shintani *et al.* 2001). The effects of leptin are mediated by the receptors similar to the cytokine receptors. Their extracellular ligand-binding domain is released to the circulation. It is possible to presume that, similarly to other cytokine receptors, the soluble leptin receptor has the ability to bind leptin and to regulate the proportion of the free and bound leptins. This can also regulate its tissue effects.

The aim of our study was to demonstrate the changes of serum ghrelin concentration in monstrously obese patients compared to lean subjects and to characterize the relationship between serum ghrelin, serum leptin and soluble leptin receptor levels.

Methods

We have examined two groups of subjects in our study. The group of obese patients consisted of eight obese patients (6 women and 2 men) aged 47±9.79 years and with the average body mass index (BMI) 40.2±13.4 $kg.m^{-2}$. The control group was composed of eight healthy volunteers (5 women and 3 men) with the age corresponding to the obese patients (51.7±4.8 years) and with the average BMI (22.7±1.3 $kg.m^{-2}$). All the patients gave their written consent with the study and the study was conducted with the approval of the Ethical Committee of the First Faculty of Medicine of the Charles University. The detailed characteristic of both groups are given in Table 1.

Table 1. Characteristics of obese patients and control lean subjects.

	Lean subjects	**Obesity subjects**
Age (years)	51.75±4.8	47.13±9.8
BMI ($kg.m^{-2}$)	22.69±1.3	40.26±13.4*

** $p<0.0001$*

Blood samples were taken early in the morning after a 12-h starvation. All subjects were weighed and measured and their BMI was calculated on the same day. The serum ghrelin, serum leptin, and soluble leptin receptor concentrations were measured in all samples.

The human ghrelin serum levels samples were determined in duplicate using the RIA kit (Phoenix Peptides, USA). The human leptin serum levels and leptin receptor serum levels were detected using the commercial ELISA kit (BioVendor. Czech Republic, Prague).

The statistical analysis was performed by SigmaStat statistical analysis software (Jandel Scientific, San Rafael, CA, USA). The means and standard deviations were calculated. The individual parameters detected in the control group and in the group of obese patients were compared using the Student's T-test for normal data distribution or by Mann-Whitney non-parametric test. The relationships between the individual parameters were evaluated using the Pearson´s or Spearman´s correlation.

Results

Both groups with comparable age differed substantially in BMI that was significantly higher in obese patients than in the control group (Table 1).

We have detected significant differences in the ghrelin serum levels, leptin serum levels, and leptin receptor serum levels (Table 2). The ghrelin serum levels were significantly lower in obese patients in comparison with the lean subjects. On the contrary, leptin serum levels were significantly higher in the control group. The soluble leptin receptor serum levels were significantly decreased in obese patients.

Table 2. Measured parameters in obese patients and control lean subjects.

	Lean subjects	**Obesity subjects**	**P values**
Ghrelin (pg. ml^{-1})	343.37±81.96	165±58.1	p<0.001
Leptin (ng.ml^{-1})	6.41±2.96	23.45±12.90	p<0.005
Soluble leptin receptor (U.ml^{-1})	21.80±4.99	7.25±3.44	p<0.0001

Table 3. The interdependence of laboratory parameters in the group of control lean subjects.

	BMI	**Ghrelin**	**Leptin**	**Leptin receptor**
BMI	≡	r = –0.109 p = 0.797	r = 0.745 p = 0.032	r = 0.081 p = 0.863
Ghrelin	r = –0.109 p = 0.797	≡	r = –0.641 p = 0.087	r = 0.254 p = 0.582
Leptin	r = 0.745 p = 0.032	r = –0.641 p = 0.087	≡	r = –0.363 p = 0.424
Leptin receptor	r = 0.081 p = 0.863	r = 0.254 p = 0.582	r = –0.363 p = 0.424	≡

BMI – body mass index, r – correlation coefficient

Table 4. The interdependence of laboratory parameters in the group of obese patients.

	BMI	**Ghrelin**	**Leptin**	**Leptin receptor**
BMI	≡	r = –0.311 p = 0.453	r = 0.735 p = 0.0379	r = –0.423 p = 0.403
Ghrelin	r = –0.311 p = 0.453	≡	r = 0.017 p = 0.968	r = 0.874 p = 0.023
Leptin	r = 0.735 p = 0.0379	r = 0,017 p = 0.968	≡	r = –0.047 p = 0.930
Leptin receptor	r = –0.423 p = 0.403	r = 0.874 p = 0.023	r = –0.047 p = 0.923	≡

BMI – body mass index, r – correlation coefficient

Furthermore, we analyzed the relationships between the particular parameters in both groups. Serum leptin levels correlated positively with BMI ($r=0.75$, $p<0.05$) in the control group of lean subjects. The serum ghrelin levels did not correlate with any other variable in our group of healthy controls (Table 3).

In the group of obese patients, serum leptin levels also correlated positively with BMI ($r=0.73$, $p<0.03$). Significant positive correlation was detected between the ghrelin serum levels and soluble leptin receptor levels ($r=0.87$, $p<0.02$) but no other significant relation was detected between other studied parameters (Table 4).

Discussion

In the present study, we examined the changes in serum ghrelin levels in patients with obesity. Ghrelin is a novel peptide hormone, which was discovered as an endogenous ligand for the receptors of growth hormone secretagogues. It is therefore potent growth hormone secretagogue (Arvat *et al.* 2000). Recent studies indicate the fact, that ghrelin levels in systemic circulation reflect mainly nutritional status and are predominantly involved in the regulation of energy homeostasis. Thus the states associated with malnutrition are accompanied by an increase in serum ghrelin levels (Otto *et al.* 2001), whereas overfeeding is accompanied by a decrease in serum ghrelin levels (Tschop *et al*. 2000). In present study we have confirmed these data and proved a significant decrease in ghrelin serum levels in seriously obese subjects which was comparable to that seen in healthy lean control subjects.

These alterations are in some respect the counterpart to the previously reported changes in leptin serum levels, which are increased in obesity (Ostlund *et al.* 1996, Haluzík *et al.* 1999b). Both peptides are thought to act at least in part through the changes in neuropeptide Y (NPY) secretion (Tomaszuk *et al.* 1996). This fact leads us to study the relationship between ghrelin and leptin serum levels in obese subjects. Our study confirmed that there is a significant inverse relationship between ghrelin and leptin serum levels, but it did not achieve statistical significance. It is well established that leptin is an anorexigenic peptide, which fails to suppress food intake in obese subjects because of the resistance of hypothalamic centers to leptin (Banks *et al.* 1996). This fact allows us to hypothesize that ghrelin might be able to increase the appetite and food intake and therefore is suppressed in patients with obesity. Similarly, this decrease fails to cause a reduction in food intake.

Furthermore, we analyzed the changes of soluble leptin receptor in obese subjects. Soluble leptin receptor corresponds to the extracellular domain of the long isoform of leptin receptor. It was found in high amounts in hypothalamic structures important for food intake regulation (Mantzoros and Moschos 1998). It could also modify tissue effects of leptin by decreasing its unbound fraction. In the present study decreased serum levels of soluble leptin receptor were found in systemic circulation of obese subjects. The importance of these changes is not completely understood, but a decrease in soluble leptin receptor serum levels might be a certain compensation of leptin resistance in obese subjects by increasing the fraction of free unbound leptin.

We can conclude that serum ghrelin levels are decreased in obese patients. This decrease together with the increase in serum leptin levels probably fails to suppress appetite in obese patients. We can also speculate that observed decline in serum soluble leptin receptor levels could be a certain compensatory mechanism to the leptin resistance in obese subjects by increasing bioavailability of leptin. Further studies have to be addressed to elucidate the physiological role of ghrelin in the regulation of food intake and that of nutritional status. It is possible that detailed understanding of mechanisms involved in these processes can lead to the development of strategies in therapy of such serious disorders as obesity and probably also treatment of disorders associated with decreased food intake and malnutrition.

Acknowledgements

This experiment was supported by the grants of IGA MHCR No.NB/7099-4, No.NB/5455-3 and No. NB/6663-3.

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